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USE OF UNICELLUAR ALGAE FOR EVALUATION OF POTENTIAL AQUATIC CONTAMINANTS

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals, "Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

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Several species of marine and freshwater algae were used in bioassays to determine the effects of potential aquatic contamination which might result from the use of hydrazine and shale derived fuel propellants. Techniques are described for both chronic and spill types of exposure and the results are each are discussed. Compounds evaluated included hydrazine, monomethylhydrazine, unsymmetrical dimethylhydrazine and shale JP-8.

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PREFACE

This is the Fifth Annual Report of work performed under the Air Force Contract AF33615-76-C-5005 and covers the period June 1, 1979 to May 31, 1980. The project is entitled "Use of Unicellular Algae for Evaluation of Potential Aquatic Contaminants." Research was conducted by the Water Resources Laboratory, School of Engineering, University of California, Irvine. The investigation was designed to expand the knowledge of toxic biostimulatory responses of unicellular algae to hydrazine propellants and synthetic jet fuels including shale-derived JP-8. This information will aid Air Force personnel in assessing the environmental impact of compounds which may be released into the aquatic environment.

Contract monitor was Lt. Col. C.B. Harrah, Chief, Environmental Quality Branch of the Toxic Hazards Division, AFAMRL, Wright-Patterson Air Force Base, Dayton, Ohio. Principal investigators were Jan Scherfig, Civil and Environmental Engineering and Peter S. Dixon, Department of Ecology and Evolutionary Biology, University of California, Irvine. Mrs. Carol Justice conducted the morphological studies and Mr. Alberto A. Carrillo performed the shale oil exposure experiments.

The authors gratefully acknowledge the assistance of Mrs. D. Franks for her efforts in growth media preparation, algal bioassay monitoring, and the overall conduct of laboratory procedures of this study.

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SUMMARY

This report presents the work conducted during 1979/80 to evaluate and quantitate the relative environmental toxicity of certain space shuttle and jet propellants. These results will permit the U.S. Air Force to provide better information for the environmental review process and also allow a much more quantitative basis for selection of alternative propellants.

The specific propellants investigated were:

1. Unsymmetrical dimethylhydrazine.
2. Monomethylhydrazine.
3. Aerozine 50 -- a 50:50 mixture of hydrazine and unsymmetrical dimethylhydrazine.
4. Shale-derived JP-8 jet fuel.

The investigations were based on the use of the relatively rapid and convenient batch algal bioassay procedure as well as consideration of continuous culture results. The methods can simulate the effects of accidental spills or low level continuous discharges of the propellants investigated. The initial low cost batch algal assay is a convenient screening tool which can then be supplemented with detailed investigations such as comprehensive batch studies as well as continuous algal assay studies. Such detailed studies should only be conducted if the preliminary results indicate a potential toxicity problem exists. Results of continuous culture algal assays with other compounds have been reported in previous annual reports.

CONCLUSIONS

The following conclusions can be drawn from the investigations conducted during 1979/80:

1. Aerozine 50 showed the same environmental toxicity as hydrazine, the most toxic of its two components.
2. Unsymmetrical dimethylhydrazine and monomethylhydrazine above their safe concentrations cause severe morphologic changes in internal cell structure based on both scanning and transmission electron microscopy.
3. Scanning electron microscopy is a valid, routine tool to evaluate algal bioassays. Scanning electron microscopy observations of the algae used in the bioassays correlate very well with growth parameter results.

4. Toxic concentrations of the hydrazines are generally so low that very accurate low volume pumping systems are needed to conduct continuous culture algal assays to stimulate low level continuous discharge. Such pumps are now available and may be used next year.

5. Shale-derived JP-8 contains significant and highly toxic water soluble components.

6. The morphologic changes in the organisms exposed to hydrazine compounds were very different from those cells exposed to shale-derived JP-8 although all fuels are equally toxic to the test organisms based on cell growth.

RECOMMENDATIONS

Based on the conclusions derived in this investigation the following recommendations are made:

1. Additional efforts should be made to improve GC/purge-trap procedure to analyze the water soluble fraction of petroleum or shale based fuels.

2. More accurate continuous culture algal assays should be conducted using the new very low volume pumping systems now available. Results from such assays will aid the Air Force in determining appropriate limits for continuous discharge of low level concentrations of specific propellants.

3. Investigations should continue for other jet fuels including JP-4, JP-8 with and without additives, as well as JP-10 to determine whether the toxicity for these fuels is similar to the effects observed from the shale-derived JP-8.

4. Qualitative and quantitative evaluations of the specific compounds which cause the toxicity of shale-derived JP-8 (and other jet fuels) should be emphasized using liquid chromatography, GC/MS, and other appropriate techniques.

SUMMARY OF PREVIOUS RESEARCH

During the previous 5 years of research, major emphasis has been concerned with evaluating toxic potential of compounds used or available for use for Air Force operations. To bring our past research efforts into focus a brief summary of the results of testing on the toxic effect of test compounds on S. capricornutum is illustrated on Table 1.

In terms of toxic potential, from this summary we may conclude that:

TABLE 1. SUMMARY OF THE EFFECT OF TEST COMPOUNDS ON S. CAPRICORNUTUM COMPILED IN PREVIOUS ANNUAL REPORTS (1975-79)

TEST COMPOUND	YEAR(S) OF ANALYSIS	SC(PPT)*	EC_{50}(PPT)*	PERCENT** GROWTH OF CONTROL
LUBE ADDITIVES:				
P, p'dioctyldiphenyl-amine	1975	N.M.	N.M.	100% (no effect)
Phenyl-α-naphthylamine	1975	N.M.	N.M.	50%
JET FUELS:				
RJ-4	1975	N.M.	N.M.	90%
RJ-5	1975	N.M.	N.M.	100% (no effect)
HYDRAZINES:				
Hydrazine	975-79	.001-.005	.013-.041	N.M.
UDMH	1975-78	.50	4.7-8.0	N.M.
MMH	1975-78	.001-2.00	.270	N.M.
SDMH	1975	N.M.	10.0	50%

** SC and EC_{50} values where taken as 1 part test compound to 1000 parts 100 percent SAAM or high nutrient medium. All SC and EC_{50} values used were taken during day 6 of testing and based on cell number.

** Percent growth of control values were based on cell number and taken from assays of 100 percent SAAM or high nutrient medium during day 6. Test compound concentration of assays used to determine values of percent growth of controls listed are:

Lube additives:	100 mg/ℓ
Jet fuels:	100 mℓ/ℓ
Hydrazines:	10 μℓ/ℓ

N.M.: not measured.

a) Of the two lube additives tested P, p'dioctyldiphenylamine is the more satisfactory compound.

b) Of the two jet fuels tested RJ-5 is the more satisfactory compound although both have limited toxic influence.

c) All hydrazines are at least moderately toxic and hydrazine and MMH are highly toxic.

This summary by no means encompasses the whole scope of research conducted and described in previous annual reports. Efforts directed towards improved testing protocol, alternate toxicity measurement methods (Warburg Experiments), alternatives in test simulation (bottle or continuous cultures), component separation and analysis by gas-chromatography, and many other topics are described in detail in previous annual reports.

INTRODUCTION

The need for specific information regarding the potential short and long term toxicity of the many potential fuels and chemicals used by the U.S. Air Force has increased in recent years. This increase is caused by the adoption of governmental legislation extending stricter controls on the introduction of chemicals and toxic wastes in the general aquatic environment as well as long term effects on specific water bodies such as lakes and groundwater basins.

Both the hydrazine propellants and the alternative jet fuels such as shale-derived JP-8 as well as JP-10 have a potential for such contamination and their effects on different aquatic ecosystems must therefore be determined.

Bioassays using unicellular algae provide a quantitative, rapid, and economical method to determine such effects over a wide range of water quality conditions. Algal assays are convenient to use because the equipment needed to conduct such tests on a routine batch basis is relatively simple. This enables the U.S. Air Force to conduct preliminary screening of a wide range of compounds. Only those which show statistically significant toxic or biostimulatory effects need be tested further using detailed batch and continuous algal assays.

Since unicellular algae represent the first trophic level in the aquatic food chain they may also be used to evaluate the possibility of bio-accumulation of potential toxic compound.

OBJECTIVES

Research objectives for the past year have been directed towards completing the determination of toxic and/or biostimulatory effects

of three hydrazine compounds on several species of unicellular green algae under different natural water conditions with emphasis on changes in cell morphology. The second major effect has centered around the evaluation of shale-derived JP-8.

Both freshwater and marine bioassays were conducted under varying test conditions to simulate a range of aquatic ecosystems, such as oligotrophic lakes, eutrophic lakes, lakes of intermediate trophic status, estuaries and the open sea. The overall goals have been to provide information about relative safety of the compounds for environmental impact statements and determine threshold limits under which the Air Force can operate within the requirements of the National Environmental Policy Act.

Specific Objectives

1. Determine the safe concentration (maximum 'no effect' concentration) for each of the compounds under the various test conditions. The safe concentration (SC) is defined as the highest concentration of test compound that can be administered without causing a detectable difference in cell mass and cell volume on the sixth day of the bioassay.

2. Determine the median effect concentration (EC_{50}) for the compounds under the various test conditions. The EC_{50} is that concentration of test compound which causes a fifty percent reduction in algal growth when compared to controls on the sixth day of the bioassay.

3. Determine how the SC and EC_{50} doses would be affected by changes in water quality and different algal species.

4. Evaluate the transient versus chronic effects of the test compounds to aid in preparation of more meaningful environmental impact statements.

5. Initiate detailed investigations of the type and extent of cell morphological changes caused by the different test compounds.

WORKPLAN

The workplan is divided into four main parts related to the specific objectives.

1. Completion of monomethylhydrazine (MMH), unsymmetrical dimethylhydrazine (UDMH), and Aerozine 50 evaluations under simulated fresh water conditions using Selenastrum capricornutum as the assay organism.

2. Initiation of detailed morphological studies of cell deformation induced by hydrazines and shale-derived jet fuels by means of both scanning and transmission electron microscopy.

fuels by means of both scanning and transmission electron microscopy.

3. Determination of safe concentration (SC) and effective concentration (EC_{50}) for shale-derived JP-8 jet fuels.

4. Investigation of the stability and composition of the water soluble fraction of shale-derived JP-8 under the bioassay conditions.

The workplan was established on a yearly basis with provisions for modifications to reflect changes in objectives as indicated by the results obtained during the year. Specifically, during the year there was a shift in emphasis toward more detailed electron microscopy work using transmission EM because of the significant effects observed from cell size changes resulting from exposure to MMH.

TOXICITY MEASUREMENTS AND TEST METHODS

The analytical and assay procedures have been reported in detail in the previous annual reports.[5] Only modifications and new procedures developed during the present year are discussed in this annual report.

TOXICITY MEASUREMENTS

Biological Growth Measures

The biological response to the compounds studied was determined by measurement of several aspects of algal growth activity including oxygen production rates, specific growth rates, growth (biomass) at a specific day after the start of the bioassay, and maximum biomass produced. During the early periods of this investigation extensive work was done to evaluate the applicability of methods to interpret the results obtained with each of these parameters. Based on that work it was decided that biomass and number of cells should be used to evaluate the effects of potential toxic compounds.

Initially, the biomass parameter used was the maximum standing crop. It is defined as the amount of algal growth (as cell numbers or total cell volume) obtained when algal growth had culminated. This is determined as the time when the increase in algal growth has stopped or decreased to less than five percent per day.

Measurement of maximum standing crop may be complicated when the potentially toxic compound being tested is unstable in natural waters. This problem surfaced during the studies of hydrazine compounds. Here, the instability is such that the compound will totally decompose within a few days compared with the 10 to 15 days needed to reach maximum standing crop. Thus, even though there is a

significant short-term toxic effect from hydrazines, after 10 days the net effect is sometimes biostimulatory, perhaps because the nitrogen in the hydrazines becomes available to the algae.

A similar problem occurred during the testing of the shale-derived JP-8. In this case we postulated that the reduction in toxicity during the bioassay was due to evaporation, biodegradation, or a combination thereof, of the specific toxic components of the shale-derived JP-8.

Several alternative growth parameters were considered. After evaluating the relative growth for a range of different compounds and the resulting algal growth we decided to use the biomass on the fifth, sixth, or seventh day as the growth parameter. This selection is somewhat arbitrary but reflects our experience that during this time, in general, the largest difference in biomass between treated and control cultures occurred. The decision not to select one single day was based on the observation that there was little relative difference on any of those three days. Thus the practical work relating to conduct of multiple simultaneous assays and scheduling of counting was facilitated by this flexibility.

Toxic Concentrations

Two complementary measures were selected to quantify the toxic levels of the hydrazine compounds. The first of these, the safe concentration (SC), is the maximum concentration of a hydrazine fuel which can be present without causing a statistically detectable difference in algal biomass. The second measure used is the median effect concentration (EC_{50}) which is that concentration that results in a 50 percent reduction in biomass on the sixth day of growth when compared to the control. In both cases, the concentrations are determined on the basis of Analysis of Variance and t-tests for the specific experiments combined with interpolation between concentrations of hydrazines added.

METHODS

Algal bioassays were conducted in accordance with Standard Methods (American Public Health Association, 1975) and the algal Assay Procedure: Bottle Test (United States Environmental Protection Agency, 1971) in order to determine the safe concentration (SC) and median effective concentration (EC_{50}).

Algal Bioassays

Modifications of the Algal Assay Procedure included the following:

1. A larger volume of medium was used (250 mℓ/500 mℓ flasks) in conjunction with an auxiliary aeration system.

2. Temperature control was 23 ± 3°C.

3. All compounds contained in the growth medium were added in a particular order before filtration in order to prevent iron precipitation. The order of additions was sodium bicarbonate, magnesium sulfate, calcium chloride, potassium orthophosphate (mono-H), magnesium chloride, sodium nitrate and trace metals including a chelating agent.

Algal bioassays were conducted in two steps: (1) a broad screening series and (2) a fine evaluation analysis. A preliminary series of replicate flasks containing the algal growth medium was dosed with a broad range of concentrations (e.g. from 0.001 to 10 ppm) of the test compound. Flasks were seeded with the appropriate test organism and algal growth (both total cell number and total algal volume) was monitored with an electronic particle counter (Coulter model TA II with Population accessory) until the control flasks without test compound reached the maximum standing crop. The maximum standing crop or maximum biomass is defined as having been achieved when the biomass increase is 5 percent or less per day. In this way it was possible to determine the approximate concentration range of the SC and EC_{50}. Subsequently, another series of flasks containing growth medium was dosed with this narrow concentration range of the test compound.

All flasks were seeded to an initial concentration of 1×10^6 cells/ℓ with the appropriate algal species. The test organism for freshwater bioassays was S. capricornutum. Initial investigations of marine waters used Dunaliella tertiolecta as the test organism but the differences in response between this alga and Selenastrum reported in the 1978 annual report) caused us to question whether the cell type might be responsible, Selenastrum being bounded only by the cell membrane or plasmalemma. During the current year, other unicellular algae have been tested on a preliminary basis. Algal growth was monitored as described previously and the SC and EC_{50} concentrations were determined. The Standard Algal Assay Medium (SAAM) was the growth medium for freshwater bioassays and modified Burkholder's artificial seawater (ASW) with varying SAAM levels of nitrogen and phosphorus was the medium for the marine algal assays.

Simulation of Environmental Problems

Chemicals and fuels used by the U.S. Air Force may enter the aquatic environment by two major modes. The first of these is by accidental spill which will cause an abrupt increase in concentration followed by a gradual decline through dilution and decomposition. This type of event is simulated by batch assays as described by the American Public Health Association (1975). The second mode is through a continuous or semi-continuous release as would result from a waste treatment plant which treats a waste to a given low effluent concentration. This type of system is simulated in a laboratory by means of continuous cultures that allow the

maintenance of steady concentrations over reasonable periods of time.

Algal bioassays were conducted in two steps: (1) a broad screening series and (2) a fine evaluation analysis. A preliminary series of replicate flasks containing the algal growth medium was dosed with a broad range of concentrations (e.g. from 0.001 to 10 ppm) of the test compound. Flasks were seeded with the appropriate test organism. Algal growth, both total cell number and total algal volume, of each flask was monitored with an electronic particle counter (Coulter model TA II with Population accessory).

Test Compound Concentration

Test compounds were prepared by serial dilution from the stock bottle immediately before being added to the bioassay flasks containing the algal cells. Five replicate flasks were prepared for each of the desired initial concentrations of test compound. A sample was removed from at least three of the bioassay flasks and analyzed chemically to determine whether the desired and actual concentrations were in agreement. In some cases the limit of detection for a particular compound was higher than the desired initial concentration and direct verification of the amount present was not possible. In most cases, the "desired" and "actual" initial concentrations were in very good agreement.

Analytical Methods

Hydrazines

The analytical methods used for the hydrazine (HZ, MMH, UDMH) including the modification and improvements developed during the study are described in the previous annual report (Scherfig et al., 1978).

Jet Fuels

The water soluble fractions of the jet fuels (regular and shale-derived JP-8) were analyzed by gas-chromatography (GC) and total carbon analysis (TOC).

The first step in the GC analysis consisted of purging the dissolved organic compounds from the water sample by means of a nitrogen gas stream. The purged compounds were trapped in an absorption column and subsequently desorbed by elevating the temperature. The desorbed compounds in the carrier gas then entered the injection port of the GC and the GC analysis commenced. The detailed procedure including column condition is described in the Appendix.

The analytical procedure was calibrated against hydrocarbon standards ranging from C_6 through C_{15}. Two calibration procedures were used. The first involved direct injection of individual compounds as well as mixtures of the C_6 through C_{15} dissolved in carbon disulfide. In the second procedure, the calibration mixture was added quantitatively to the water in the purge unit. The mixture was purged with nitrogen carrier gas and transferred to the trap column where it was absorbed. Recovery of the components of the mixture was then possible by a preferential desorption process, created by gradual heating of the trap, separating components when desorbed off the trap. Recoveries are noted in the Appendix and are summarized in Table 2. The recoveries range from 12 to 70 percent with the best recoveries obtained for C_8 through C_{12}.

TABLE 2. STANDARD HYDROCARBON MIXTURE RECOVERY IN PURGE AND TRAP UNIT

Compound	Weight g x 10^{-6}	Area (Ideal)	Area (Real)	% Recovery
C_6H_{14}	39.2770	5.852	1.400	23.92
C_7H_{16}	9.2055	2.449	1.063	43.41
C_8H_{18}	10.4330	2.542	1.725	70.35
C_9H_{20}	19.9560	2.649	1.643	62.02
$C_{10}H_{22}$	16.5600	2.850	1.550	54.39
$C_{11}H_{24}$	15.9560	2.697	1.400	51.91
$C_{12}H_{26}$	14.1150	2.654	1.650	62.17
$C_{13}H_{28}$	15.9560	2.697	0.840	31.15
$C_{14}H_{30}$	15.9560	2.697	0.338	12.53
$C_{15}H_{32}$	16.5700	2.552	--	--

Work is continuing to improve the recoveries in both ends of the range through modification of both the purging and desorption conditions. Because of the varying recoveries, the GC analyses were supplemented with total organic carbon analyses. This provides information about the absolute amounts of organic carbon providing a guide to maintain a consistent level of extraction of the water soluble fraction from the fuels. This also indicates whether there are significant quantities of organic carbon compounds which cannot be detected due to the particular packing material in the trap and the GC column.

Preparation of Fuel/Water Mixtures for Bioassay

A standard extraction procedure was selected to prepare a bioassay medium with a constant amount of water soluble compounds from a given fuel. It was prepared by mixing the Standard Algal Assay Medium (SAAM) with an amount of JP-8 which represents five percent by volume. The mixture was placed in separatory funnels

which were shaken for 1 minute every 2 hours during an 8 hour period. The funnels were then left to settle and separate into two layers (approximately 48 hours). The bottom (water) part represents the soluble fraction of JP-8 or shale-derived JP-8 in the standard algal assay medium (SAAM). The mixture is referred to as Standard Algal Assay Medium with soluble fraction of fuel (SAAM + SFF). In addition to this method, we also tried to extract by means of magnetic stirrers in closed bottles but the separating funnel procedure gave the most reproducible results and was therefore selected even though it was more time consuming. A range of dilutions of SAAM + SFF was then made in SAAM to determine the SC and EC_{50}.

RESULTS AND DISCUSSION

The objective of the overall investigation was to determine the potentially adverse effects of fuels which enter the aquatic environment either as the result of a spill (accident) or as low level long term continuous discharges. During this year, emphasis was placed on the spill conditions to obtain information more rapidly about the shale-derived JP-8. Continuous algal exposure studies of hydrazines were not conducted this year since the semi-continuous test reported for MMH in the previous annual report (Dixon et al., 1979) showed that the ED_{50} and SC concentrations were below the detectable limit of the analytical procedures. The concentrations were also too low to be maintained reliably with the existing chemical feed pump systems without analytical controls. New pumps have come on the market and continuous culture exposures can now be implemented in the future.

UDMH BIOASSAYS

Bioassays were conducted using target concentrations of 0.5, 1.0, 2.0, 3.0, 4.0 , and 5.0 µℓ of UDMH/ℓ SAAM. Five controls without UDMH added and five replicates for each of the above concentrations were seeded with S. capricornutum to an initial cell density of 1×10^6 cells per liter. Actual UDMH concentrations were determined at the beginning of the bioassay. Initial UDMH concentrations are shown in Table 3.

Algal growth, (cell number, total algal cell volume, and mean cell volume) was determined on growth days five and fourteen. Samples were taken for scanning and transmission electron microscopy on growth day five. Summarized algal growth data are presented in Table 4.

Safe concentration (SC) ranged from 0.5 µℓ for cell number data on growth day five to about 1.2 µℓ for total cell volume data on day fourteen. These SC values are very close to those obtained under similar experimental conditions in 1977; however, EC_{50} values were approximately half of those EC_{50} values obtained in 1977. This

could be due to a number of factors including: (a) variability of the test organism; (b) slight change in the UDMH which would not be detected by the chemical method used; or (c) differences in external environmental conditions such as temperature.

Examination of mean cell volume (MCV) data from growth days five and fourteen shows a progressive increase in cell volume with increasing UDMH concentrations. The increase in cell size is most apparent on growth day five where those cells exposed to an initial UDMH concentration of 5 µℓ/ℓ are approximately 2.5 times the size of the control cells which have not been exposed to UDMH. Samples from growth day five were taken for examination with the electron microscope.

AEROZINE 50 BIOASSAYS

Bioassays were conducted using a broad concentration range of Aerozine 50 (AZ_{50}) in order to determine the SC and EC_{50} values. Initial AZ_{50} concentrations were 0.001, 0.01, 0.10 and 1.0 µℓ/ℓ of 100 percent SAAM. Two replicate flasks for each concentration and two controls were seeded with S. capricornutum.

TABLE 3. TARGET AND ACTUAL INITIAL UDMH CONCENTRATIONS IN BIOASSAY

Flask Numbers	UDMH Concentration µℓ/ℓ	
	Desired	Analyzed
1-5	0.00	0.00
6-10	0.50	0.48 ±0.02
11-15	1.00	1.00 ±0.03
16-20	2.00	1.98 ±0.02
21-25	3.00	3.03 ±0.03
26-30	4.00	3.96 ±0.06
31-35	5.00	5.06 ±0.08

TABLE 4. GROWTH OF S. CAPRICORNUTUM IN 100% SAAM IN THE PRESENCE OF VARIOUS UDMH CONCENTRATIONS

Initial UDMH Concentration µℓ/ℓ		GROWTH DAY 5			GROWTH DAY 14		
		10^6 cells/ℓ	$mm^3/ℓ$	MCV-μm^3	10^6 cells/ℓ	$mm^3/ℓ$	MCV-μm^3
0 (controls)	$\bar{x}$	1638	66	41.0	2462	120	48.7
	s	105	5	2.9	38	11	4.0
0.48	$\bar{x}$	1656	70	42.5	2244	108	48.3
± 0.02	s	122	4	1.6	61	3	0.6
1.00	$\bar{x}$	1493	61	41.1	2290	106	46.8
± 0.03	s	70	3	1.2	160	7	0.8
1.98	$\bar{x}$	970	50	51.4	1652	88	53.4
± 0.02	s	75	3	3.3	109	8	1.2
3.03	$\bar{x}$	420	30	70.2	1128	71	62.7
± 0.03	s	91	8	8.5	23	2	1.2
3.96	$\bar{x}$	137	11	80.9	898	61	67.5
± 0.06	s	53	3	10.0	58	4	2.1
5.06	$\bar{x}$	26	2.8	107	804	54	66.7
± 0.08	s	5	0.5	26	88	5	1.7

$\bar{x}$: mean; s: Standard Deviation

Growth was monitored for a total of nine days. Algal growth data are presented in Table 5. Results from the bioassay indicate:

1) The safe concentration is >0.001 and <0.01 µℓ/ℓ.

2) The fifty percent effective concentration is >0.01 and <0.1 µℓ/ℓ.

3) AZ_{50} causes cell enlargement.

The results presented in Table 5 also show clearly the previously mentioned observation, that there is typically no significant difference between the treated and control cultures for a range of days after growth and days five through seven. This substantiates the use of a flexible counting schedule within day five through seven.

TABLE 5. GROWTH OF S. CAPRICORNUTUM IN 100% SAAM IN THE PRESENCE OF VARIOUS CONCENTRATIONS

Initial AZ_{50} Concentration µℓ/ℓ	GROWTH DAY 5			GROWTH DAY 7		
	10^6 cells/ℓ	mm^3/ℓ	MCV-μm^3	10^6 cells/ℓ	mm^3/ℓ	MCV-μm^3
0 (Controls)	2163	109	50.4	2227	10	44.9
0.001	1965	83	42.2	2208	97	43.9
0.01	1983	83	41.8	2138	99	46.3
0.10	20	1.2	60.5	29	2.3	80.6
1.0	14	0.7	46.8	16	1.8	122

Analysis of the results and comparison with the previously reported results for hydrazine in the third annual report (Scherfig et al., 1978) and the UDMH results reported above are presented in Table 6.

TABLE 6. COMPARISON OF HYDRAZINE, UDMH, AND AEROZINE 50 SAFE CONCENTRATIONS AND EC_{50} VALUES FOR S. CAPRICORNUTUM (Values in Table are the µℓ/ℓ concentrations in Batch Assays)

Parameter	Compound		
	HZ	UDMH	AZ_{50}
Safe Concentration	0.001	0.5-1.2	0.001-0.01
EC_{50}	0.03	2.2	0.01-0.1

Reference: Scherfig et al., 1978.

These results show that the toxicity of Aerozine 50 is clearly dominated by the toxicity of the most toxic component, hydrazine. Thus, Aerozine 50 appears to have the same environmental toxicological properties as hydrazine.

MORPHOLOGICAL CHANGES

The quantitative determination of growth response presented above included determination of both the number of cells and the total cell volume at various times after the beginning of each bioassay. This information allows the calculation of the mean cell volume for exposed algal cells. As the analytical techniques became

more refined the significant increase in the mean cell volume following exposure to MMH and UDMH was more apparent. Consequently, we decided to investigate the morphologic changes in detail.

MMH Induced Morphologic Changes

The results of a special MMH exposure experiment conducted to investigate morphologic changes are presented in Table 7.

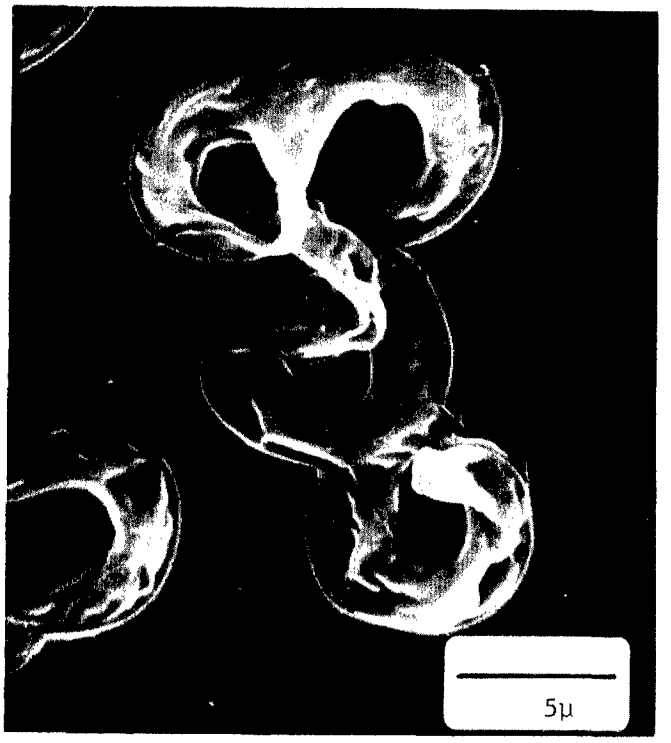
Growth day five (Table 7) shows clear evidence of cell enlargement. Samples taken from both the control flasks and the MMH dosed flasks were examined using scanning electron microscopy. Representative results from the electron microscope scans are presented in Figure 1. Figure 1-A shows typical cells from the control flasks without MMH added and Figure 1-B shows cells from flasks dosed with 0.6 μℓ of MMH per liter of growth medium. Cells from the control flasks appear somewhat collapsed in that the cytoplasm is pulled away from the cell walls but the cell walls appear to be intact. Cells from the flasks dosed with 0.6 μℓ of MMH per liter of growth medium look abnormal, both with respect to size and cell wall. They are large compared with the control cells and the cell walls seem to have disintegrated or split and the cytoplasm appears to be oozing from the cells.

TABLE 7. EFFECT OF MMH ON MEAN CELL VOLUME GROWTH OF S. CAPRICORNUTUM IN 33% SAAM AFTER FIVE DAYS OF GROWTH

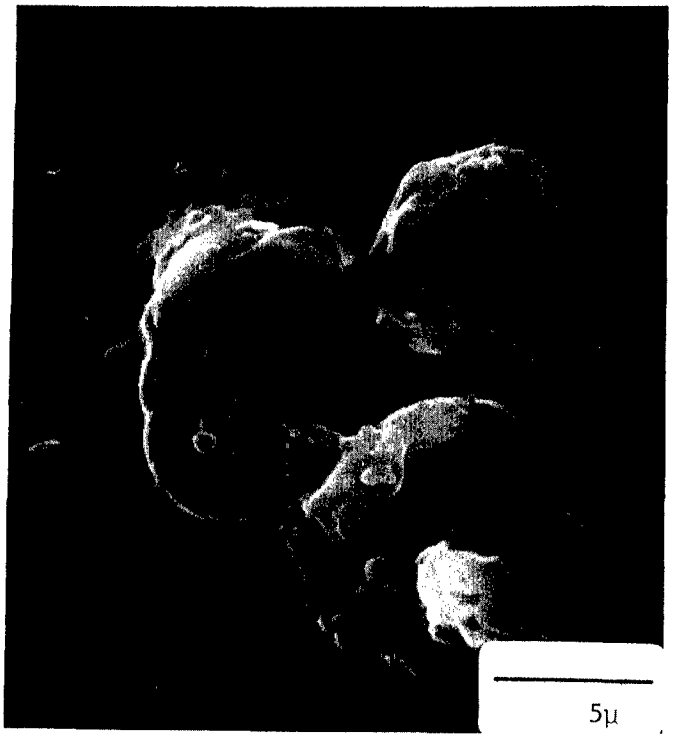
MMH Concentration μℓ/ℓ		Cell Number 10^6 cells/ℓ	Algal Volume $mm^3/ℓ$	Mean Cell Volume μm^3
Control	$\bar{x}$	1776	70.7	39.8
(no MMH)	s	46	0.6	0.7
0.2	$\bar{x}$	1290	49.6	38.6
	s	59	2.1	2.4
0.4	$\bar{x}$	170	10.0	58.0
	s	60	3.8	1.9
0.6	$\bar{x}$	34.5	2.4	71.2
	s	5.6	0.6	1.8
0.8	$\bar{x}$	47.6	2.8	63.4
	s	26.6	1.1	13.1

$\bar{x}$: mean; s: Standard Deviation

The control cells in Figure 1-A did not look as well rounded as normally observed; thus even though there was a clear distinction between the control and exposed cells, an investigation of the reason for the morphologic distortion of the control cells was conducted.



A. S. CAPRICORNUTUM FROM FLASKS (CONTROL)



B. S. CAPRICORNUTUM FROM 0.6 μℓ/ℓ MMH DOSED FLASKS

FIGURE 1 SCANNING ELECTRON MICROGRAPHS SHOWING EFFECTS OF MMH ON CELL MORPHOLOGY

Several possible causes were investigated. Ultimately we found that the morphological change in the control cells had been caused by a lower than standard concentration of salts in the assay medium. Cells grown in a freshly prepared medium with identical salt concentrations and differing only in the amount of nutrients (nitrogen and phosphorus) are shown in Figures 2A and 2B for the 33 percent and 100 percent SAAM. Both figures show the normal, firm appearance, indicating normal turgor.

UDMH Induced Morphological Changes

Exposure of S. capricornutum to various concentrations of UDMH produced results similar to, and even more distinct morphological changes than those observed from the MMH exposure reported above.

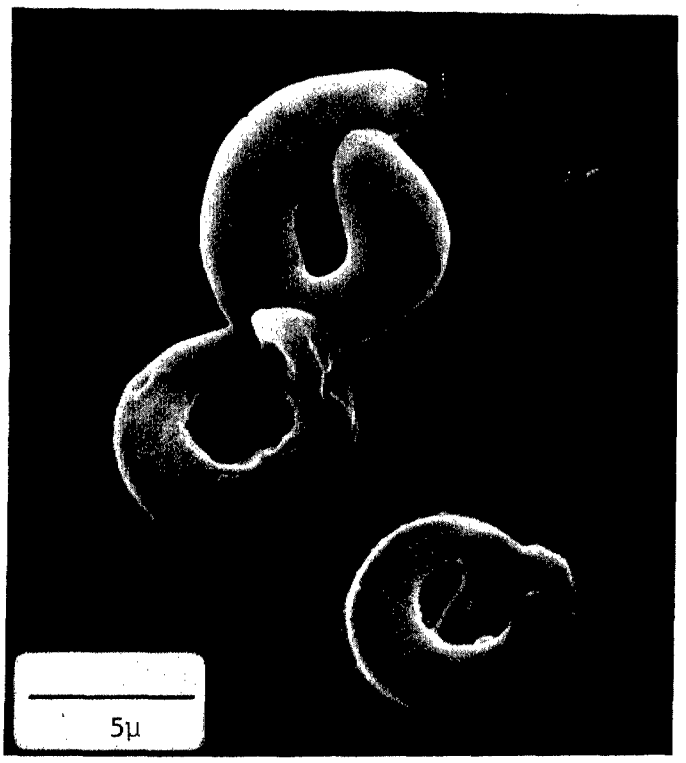
The increases in mean cell volume resulting from the UDMH exposure are summarized in Table 8.

TABLE 8. MEAN CELL VOLUME INCREASE IN S. CAPRICORNUTUM FROM UDMH EXPOSURE

Initial UDMH Conc. µℓ/ℓ	Growth Day 5 MCV-μm^3	Growth Day 14 MCV-μm^3
control (0)	41	49
0.48	43	48
1.00	41	47
1.98	51	53
3.03	70	63
3.96	81	68
5.06	107	67

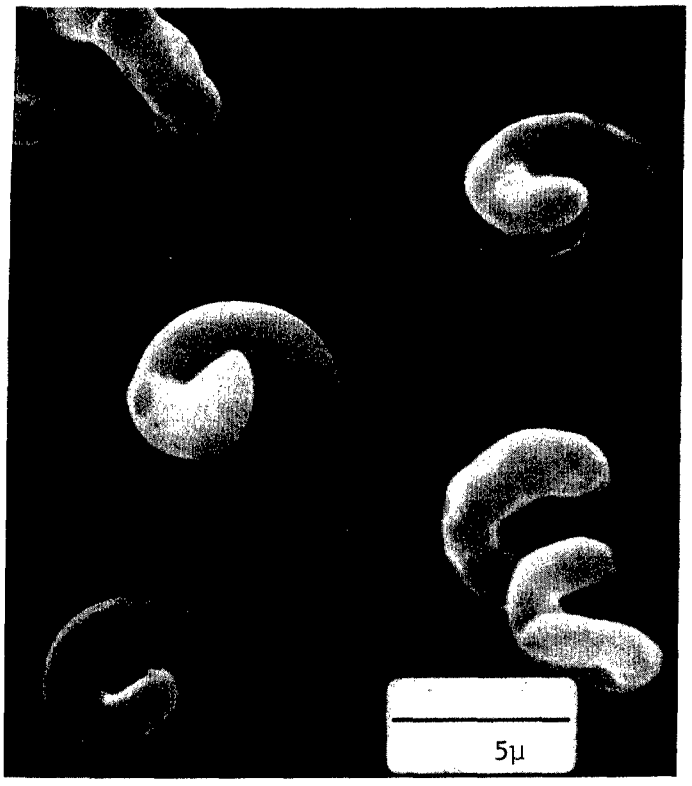
The scanning electron micrographs in Figure 3 show cells from (A) control flasks and (B) flasks containing 0.5 µℓ UDMH per liter of 100 percent SAAM. Cells from the control flasks appear slightly more coiled but this may be an artifact of the drying procedure. Cells from the 0.5 µℓ/ℓ UDMH dosed flasks are, by visual observation, very similar in size and shape to the control cells. This agrees with growth data obtained from the Coulter Counter which indicates that 0.5 µℓ/ℓ is the "no effect" Safe Concentration. Therefore, one would not expect to be able to distinguish between untreated and 0.5 µℓ/ℓ dosed cells.

Micrographs of cells from the 1.0 µℓ/ℓ dosed flasks are not included; however, these cells were relatively normal appearing with respect to size, shape and "firmness." An occasional "thin" cell was seen but there was no attempt made to quantify this number. Data from the Coulter Counter showed that on growth day five there



A. HEALTHY APPEARING CELLS OF S. CAPRICORNUTUM GROWN IN THE NEW BATCH OF 33% SAAM: GROWTH DAY 5.

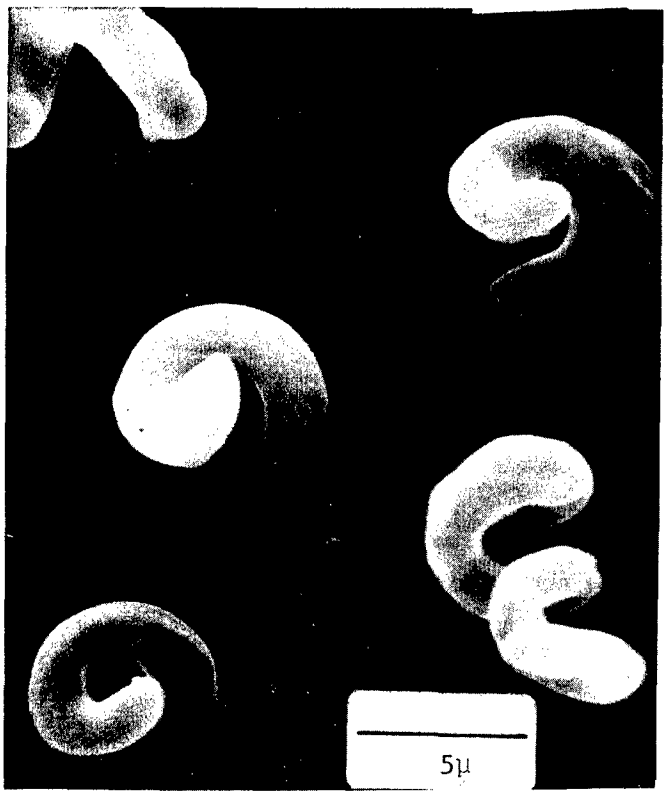
NOTE THE PLUMP, FIRM APPEARANCE OF THE CELLS, AN INDICATION OF NORMAL TURGOR OF THE CELLS.



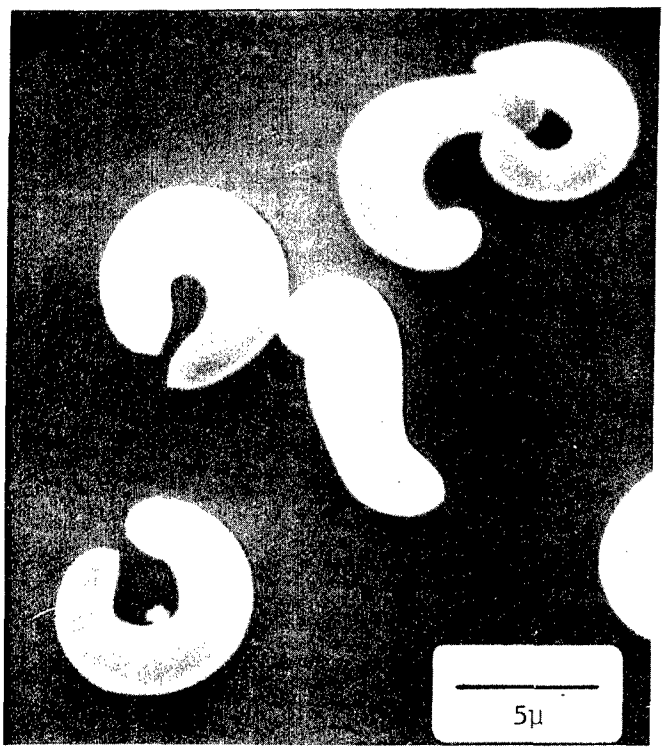
B. HEALTHY APPEARING CELLS OF S. CAPRICORNUTUM GROWN IN 100% SAAM.

THESE CELLS ALSO APPEAR WELL FILLED OUT AND MAINTAIN THE ALMOST COILED APPEARANCE WHICH IS COMPLETELY LACKING IN THOSE CELLS SHOWN IN FIGURES 1A & 1B.

FIGURE 2. SEM SHOWING NORMAL CONTROL CELLS

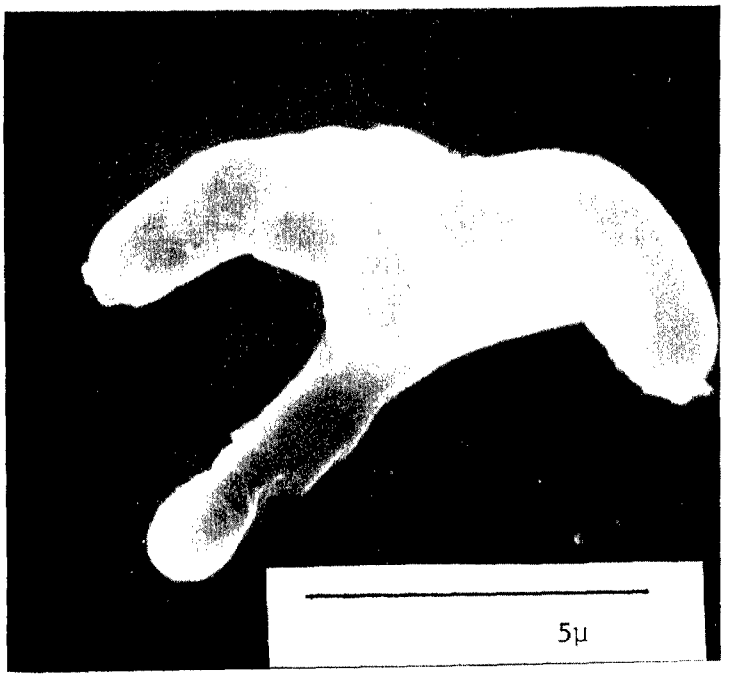


A. S. CAPRICORNUTUM CELLS GROWN IN 100% SAAM. CELLS ARE NORMAL WITH RESPECT TO SIZE AND SHAPE.



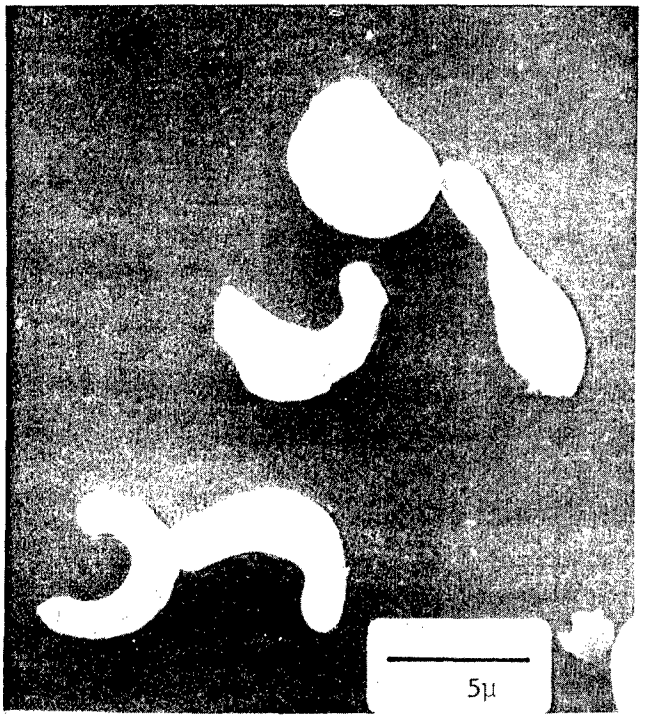
B. S. CAPRICORNUTUM CELLS GROWN IN 100% SAAM WITH AN INITIAL CONCENTRATION OF 0.5 μℓ/ℓ UDMH. THIS IS A 'NO EFFECT' OR SAFE CONCENTRATION.

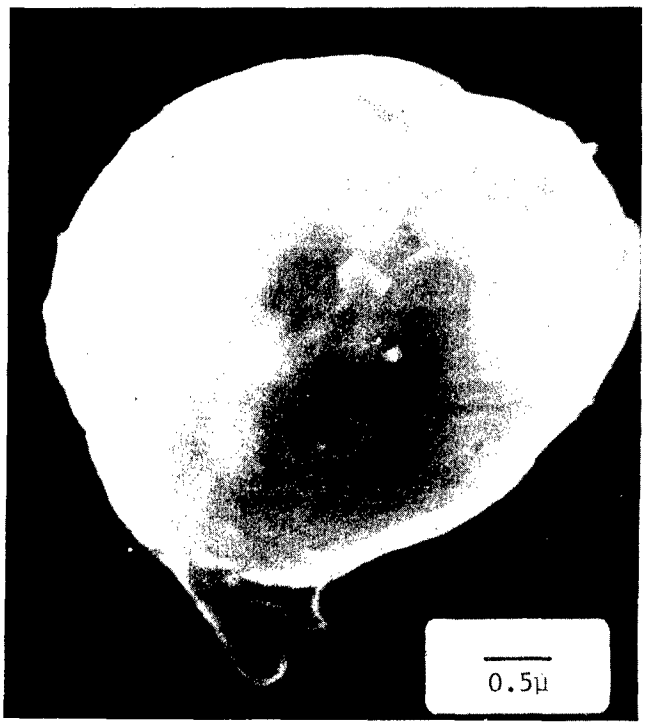
FIGURE 3. EFFECT OF 0.5 μℓ/ℓ UDMH ON CELL MORPHOLOGY



TYPICAL S. CAPRICORNUTUM CELLS GROWN IN 100% SAAM WITH AN INITIAL DOSE OF 2.0 μℓ/ℓ UDMH ADDED

FIGURE 4. EFFECT OF 2.0 μℓ/ℓ UDMH ON CELL MORPHOLOGY





S. CAPRICORNUTUM CELLS GROWN IN 100% SAAM WITH AN INITIAL DOSE OF 3.0 μℓ/ℓ UDMH ADDED. MOST CELLS ARE EXTREMELY ABNORMAL IN APPEARANCE.

FIGURE 5. EFFECT OF 3.0 μℓ/ℓ UDMH ON CELL MORPHOLOGY

KEY: CP - CYTOPLASMIC MATERIAL
G - GOLGI
P - PYRENOID BODY
N - NUCLEUS
NE - NUCLEOLUS
S - STARCH
M - MITOCHONDRIA
CW - CELL WALL

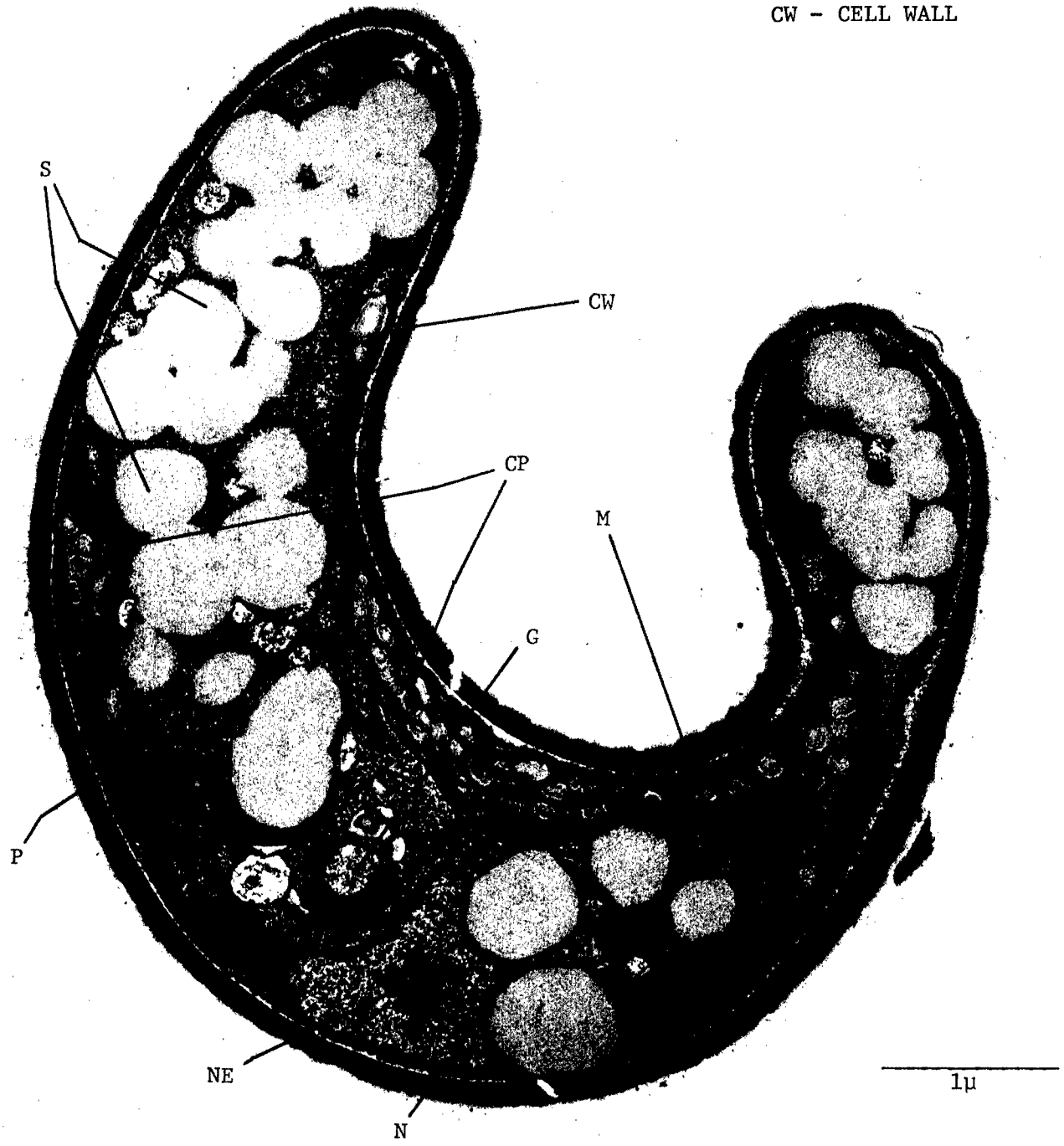


FIGURE 6. MORPHOLOGY OF S. CAPRICORNUTUM CONTROL CELLS (NO UDMH EXPOSURE)

were statistically fewer cells than in the control flask but the mean cell volume was the same.

Micrographs in Figure 4 show cells grown in the presence of an initial dose of 2.0 µℓ/ℓ (1.98 µℓ/ℓ by analysis) UDMH. These cells are abnormal in appearance, having multiple projections such as the one depicted in Figure 4B.

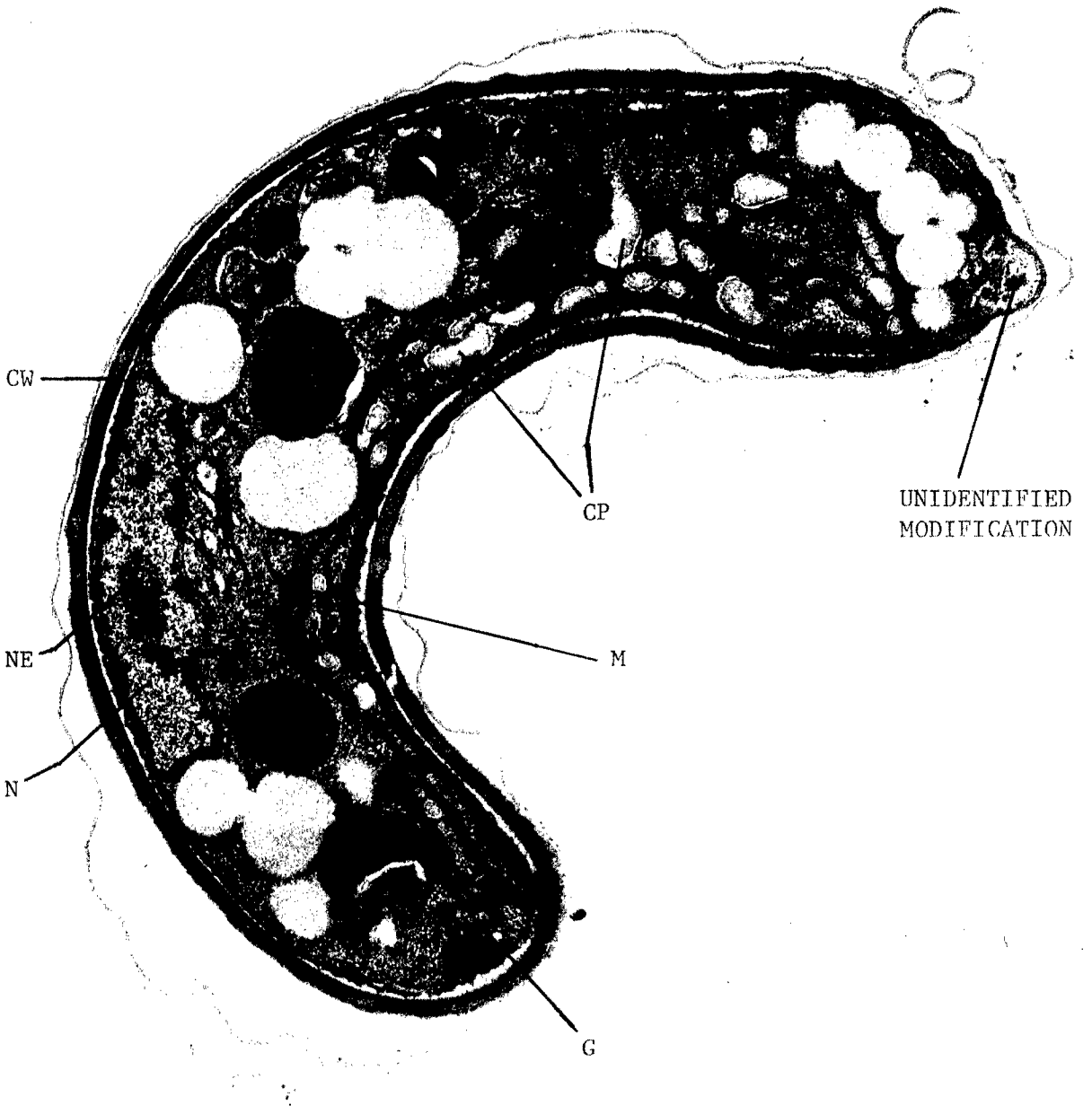
Figure 5 shows cells grown in the presence of an initial dose of 3.0 µℓ UDMH. Many gourd shaped and round cells were observed. Although some cells appeared smaller than normal, the high number of round cells must have contributed significantly to the larger mean cell volume obtained with the Coulter Counter. Transmission electron micrographs were prepared from the same samples used for scanning electron microscopy. There were no observable differences with either scanning or transmission electron microscopy between untreated control cells and those exposed to 0.5 µℓ/ℓ UDMH. A lateral section of a normal cell is shown in Figure 6. The overall impression of normal cells is that of good, well defined cell organization when compared with Figure 7 which shows cells exposed to 2 µℓ/ℓ UDMH. Structures visible in the normal cell include starch or lipid material (s), mitochondria (m), pyrenoid body (p), nucleus (n), nucleolus (ne) and Golgi (G). Chloroplast material is well organized and under greater magnification can be observed with the lamellae or bands usually composed of stacks of 3 thylakoids.

By comparison, chloroplast material in cells exposed to 2 µℓ/ℓ UDMH (Figure 7) appears disorganized with lamellae randomly arranged instead of in neat partitions. Greater magnification shows thylakoids are often not stacked at all. The ability of thylakoid membranes to stack may be affected by gene mutation and environmental conditions. In general, there appears to be a lack of definition in the exposed cells which is probably caused by a degeneration of the internal membranes. This can also be observed in the outer cell structure. In Figure 6, the cell wall (cw) is firmly attached to the plasmalemma and the cytoplasmic membrane is visible as an intact white line surrounding the cytoplasmic contents. In Figure 7, the cell wall is not firmly attached to the plasmalemma and the cytoplasmic membrane appears to be fuzzy.

JET FUELS

During the project year, detailed work was resumed on the evaluation of the environmental effects of a range of fuels beginning with implementation of analytical routines to deal with the two types of JP-8, JP-9, and JP-10. This work continues previous efforts on RJ-4 and RJ-5 (Scherfig and Dixon, 1975).

The first experiment was conducted with shale-derived JP-8 (SD-JP8). A mixture of SAAM plus 5 percent (by volume) of SD-JP-8 was prepared and stirred for 48 hours to allow the water soluble components of SD-JP-8 to dissolve in the algal growth medium. The



KEY: CP - CYTOPLASTIC MATERIAL
G - GOLGI
N - NUCLEUS
NE - NUCLEOLUS
M - MITOCHONDRIA
CW - CELL WALL

FIGURE 7. MORPHOLOGY OF S. CAPRICORNUTUM CELLS AFTER EXPOSURE TO 2μℓ/ℓ UDMH

aqueous and fuel layers were then separated and a growth experiment was initiated using the SAAM containing the water soluble components of SD-JP-8.

The results are summarized in Table 8 and show that the water soluble components from 5 percent (by volume) of SD-JP-8 were presented in signficiant concentrations and were of such high toxicity as to totally prevent growth of test organisms.

Based on these results, replicate experiments were conducted with reduced concentrations of the water soluble fraction of SD-JP-8 prepared by blending SAAM with SAAM containing the water soluble fraction from 5 percent (by volume) of SD-JP-8. This approach was selected to assure a consistent composition of the water soluble fraction of SD-JP-8. An alternative would have been to extract from varying percentages of SD-JP-8. However, preliminary tests had indicated that the solubility varied (based on total organic carbon analyses) and there was no assurance that there would not be a difference in the proportions of the individual components in the water soluble fraction of different mixtures.

TABLE 9. GROWTH OF S. CAPRICORNUTUM IN THE PRESENCE OF THE WATER SOLUBLE FRACTION FROM 5% BY VOLUME OF SHALE-DERIVED JP-8 IN SAAM

		GROWTH DAY 6			GROWTH DAY 10		
Growth Medium	Rep. No.	10^6 cells/ℓ	$mm^3/ℓ$	MCV-μm^3	10^6 cells/ℓ	$mm^3/ℓ$	MCV-μm^3
SAAM	1	6260	239	52.6	5851	295	50.4
	2	5767	293	50.8	6528	321	49.2
	3	5409	271	50.0	6249	299	47.6
	$\bar{x}$	5812	298	51.1	6209	305	49.1
	s	427	29	1.3	340	141	1.4
SAAM	1	77	3.7	48.1	94	5.3	56.0
+	2	84	5.1	60.1	111.7	4.2	37.4
Water	3	87	3.7	43.0	128.6	5.1	39.4
Soluble Fraction of Shale	$\bar{x}$	84	4.8	56.1	100.0	4.3	43.4
JP-8	s	19	2.2	12.7	21.8	0.9	8.2

$\bar{x}$: mean; s: Standard Deviation

The results of the exposure of S. capricornutum of varying amounts of SD-JP-8 are summarized in Tables 9 and 10. Results of algal growth in terms of cell numbers presented in Table 10

indicates a 6 day EC_{50} concentration, the lowest concentration of water soluble fraction that creates a fifty percent reduction of cell number compared to the control, of between 10 and 50 percent water soluble fraction of SD-JP-8. Table 10 also indicates a 6 day SC or 'No Effect' concentration, the lowest concentration of water soluble fraction that creates no significant effect in cell mass of volume, of between .1 and 1 percent water soluble fraction of SD-JP-8.

TABLE 10. GROWTH OF S. CAPRICORNUTUM IN THE PRESENCE OF DIFFERENT AMOUNTS OF WATER SOLUBLE FRACTION OF SD-JP-8 (ALL VALUES BASED ON 3 REPLICATES)

Growth Media Proportions SAAM with Water Sol Frac. of SD-JP-8[a])	SAAM		Growth Day 6			Growth Day 8		
			10^6 cells/ℓ	$mm^3/ℓ$	MCV μm^3	10^6 cells/ℓ	$mm^3/ℓ$	MCV μm^3
50 :	50	Mean	1027	39.1	38.6	5288	219	41.3
		St.Dev	331	9.9	3.1	158	19.1	2.4
10 :	90	Mean	4324	168	38.8	5915	258	43.6
		St.Dev	428	13.3	0.9	251	6.5	0.8
5 :	95	Mean	4559	183	40.2	6095	269	4.66
		St.Dev	114	4.2	0.5	110	5.1	1.2
1 :	99	Mean	4958	189	38.2	6353	275	43.3
		St.Dev	120	9.3	2.8	227	2.0	1.4
0.1 :	99.9	Mean	4969	178	35.9	6161	259	42.1
		St.Dev	110	2.3	0.4	97	5.0	0.2
0 :	100	Mean	4853	176	36.2	6322	268	42.4
(control)		St.Dev	182	7	0.4	276	11	0.4

[a] Note that the toxic effect appears to decrease after day six. A separate experiment was performed to determine whether this decrease was due to evaporation or biodegradation. Assay flasks were filled with SAAM plus the water soluble fraction from five percent (by volume) of SD-JP-8. The results in Table 11 show that both sets of assay flasks (with and without aeration required for CO_2/pH control is the standard procedure) lose about 25 percent of the total organic carbon within the first 24 hours and up to one-third is lost after three days but with only a small change after the second day.

Attempts to identify the volatilized components by detailed GC evaluations are in progress.

SD-JP-8 Induced Morphologic Changes

Scanning electron micrographs of cells grown in control flasks (no JP-8), 50:50, and 5:95 mixtures of SAAM with the water soluble fraction of SD-JP-8 were prepared. The cells for the scans were taken from the experiment reported in Table 10.

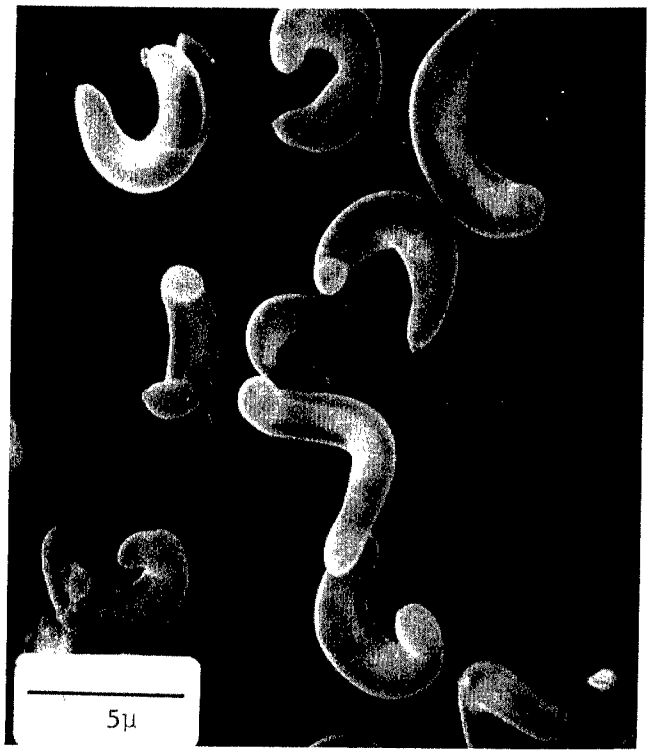
These scans show no observable difference between control cells grown in regular SAAM (Figure 2) and those grown in the 5:95 mixture (Figure 8-A). These observations are consistent with the numerical results presented in Table 10. Cells grown in 50:50 mixture are generally not as curved as the control cells and have a blunted tip at one end of the cell. Some cells are comma-shaped instead of the usual crescent shape. An enlarged view of two cells with blunted tips after growth in the presence of the water soluble fraction of SD-JP-8 is shown in Figure 8B.

TABLE 11. TOTAL ORGANIC CARBON (TOC) DECREASE OF WATER SOLUBLE FRACTION OF SD-JP-8 WITH TIME

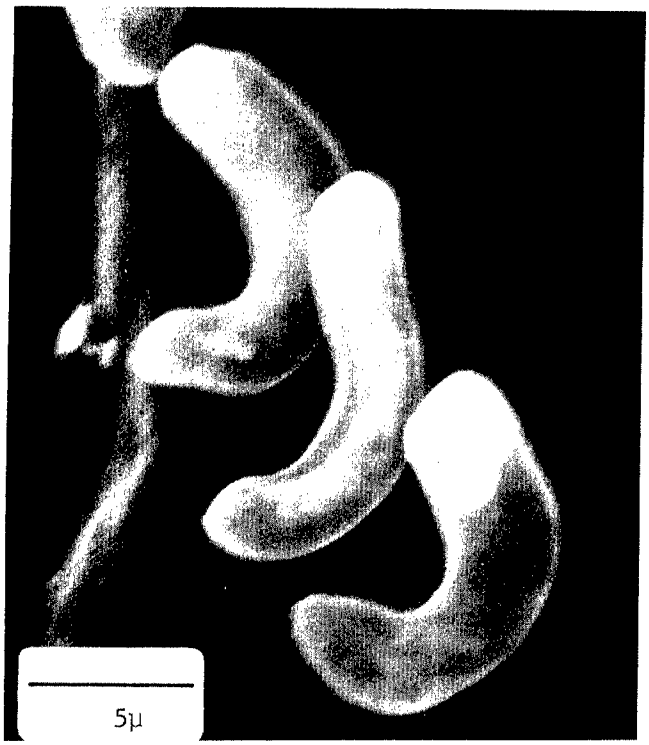
Time (Hours)	Without Aeration		With Aeration	
	Mean Conc. mg TOC/ℓ	Percent Decrease	Mean Conc. mg TOC/ℓ	Percent Decrease
0	16.3	0	12.0	0
4	14.0	14	-	-
6	13.6	17	9.5	21
27	11.6	29	9.3	23
53	10.9	33	-	-
72	10.8	34	-	-

Transmission electron microscopy was also conducted on the cells grown in the presence of the water soluble fraction of SD-JP-8.

There were no observable differences between control cells (Figure 6) and those exposed to the 5:95 medium (Table 9). Algal growth data also indicate that this is a safe dose. An unexposed cell is shown in Figure 6 while Figure 9 shows a cell exposed to a mixture of SAAM with the water soluble fraction of SD-JP-8 and regular 50:5 SAAM. Lamellae are more or less parallel to each other and thylakoids are stacked in triplicate. An improved fixation procedure was used for these cells than was used for cells exposed to UDMH (Figures 5, 6 and 7). The slightly more serrated aspect of the cell wall appears to be a result of fixation and microtome



A. S. CAPRICORNUTUM GROWN IN A 5:95 MIXTURE OF SAAM WITH THE WATER SOLUBLE FRACTION OF SD-JP-8. CELLS ARE NORMAL.



B. S. CAPRICORNUTUM GROWN IN A 50:50 MIXTURE OF SAAM WITH THE WATER SOLUBLE FRACTION OF SD-JP-8. NOTE BROKEN TIPS OF TWO UPPER CELLS AND LESS PRONOUNCED CRESCENT SHAPE.

FIGURE 8. EFFECT OF WATER SOLUBLE FRACTION OF SD-JP-8 ON S. CAPRICORNUTUM MORPHOLOGY

NOTE CHANGED CELL WALL AT ENDS.
SIMILAR TO THAT SHOWN BY
SCANNING E.M.

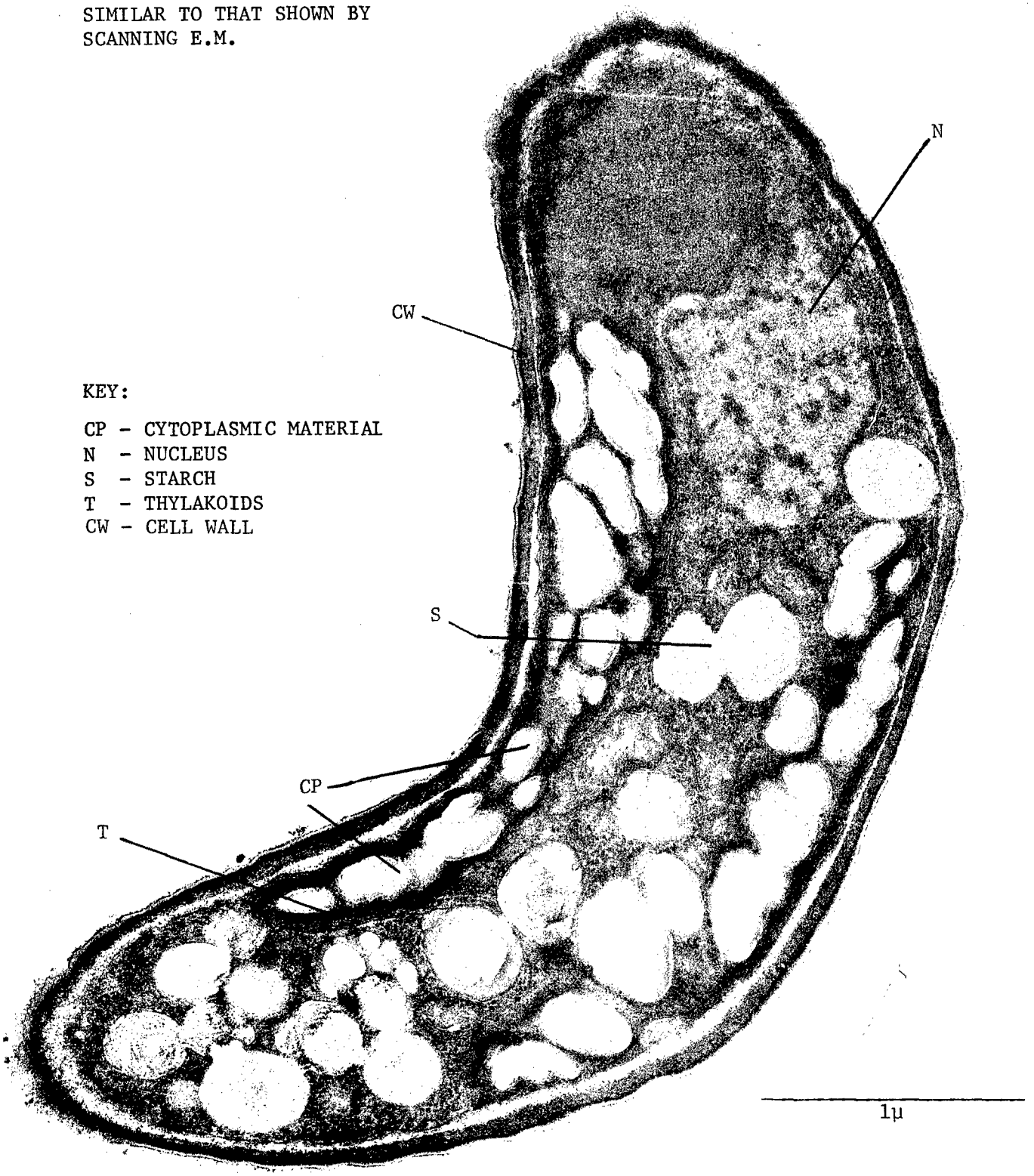


FIGURE 9. MORPHOLOGY OF CELLS EXPOSED TO WATER SOLUBLE FRACTION OF SHALE-DERIVED JP-8

sectioning since scanning micrographs show that cell walls are smooth. Internal cell structure generally shows more clearly with this Fluter-Pipes fixation. Detailed examination of Figure 9 shows that there is a clear change in the cell wall at both ends of the cell while the remainder of the cell wall appears normal. This is consistent with the results of the scanning electron microscopy results shown in Figure 8.

CONCLUSIONS

The following conclusions can be drawn from the investigations conducted during 1979/80:

1. Aerozine 50 showed the same environmental toxicity as hydrazine, the most toxic of its two components (hydrazine and unsymmetrical dimethylhydrazine).

2. Unsymmetrical dimethylhydrazine and monomethylhydrazine above their safe concentrations cause severe morphologic changes in internal cell structure based on both scanning and transmission electron microscopy.

3. Scanning electron microscopy is a valid routine tool to evaluate algal bioassays. Scanning electron microscopy findings of the algae used in the bioassays correlate very well with growth parameters results.

4. Toxic concentrations of the hydrazines are generally so low that very accurate low volume pumping systems are needed to conduct continuous culture algal assays to simulate low level continuous discharge. Such pumps are now available and may be used next year.

5. Shale-derived JP-8 contains significant levels of highly toxic water soluble components.

6. The morphologic changes produced by hydrazine compounds and by shale-derived JP-8 were very different although all compounds are equally toxic to the test organisms based on cell growth.

RECOMMENDATIONS

Based on the conclusions derived in this investigation the following recommendations are made:

1. Additional efforts should be made to improve the GC-purge-trap procedure to analyze the water soluble fraction of petroleum or shale based fuels.

2. More accurate continuous culture algal assays should be conducted using the new very low volume pumping systems now

available. Results from such assays will aid the Air Force in determining appropriate limits for continuous discharge of low level concentrations of specific propellants.

3. Investigations should continue for other jet fuels including JP-4, JP-8 with and without additives as well as JP-10 to determine whether the toxicity from these fuels is similar to the effects observed from the shale-derived JP-8.

4. Qualitative and quantitative evaluations of the specific compounds which cause the toxicity of shale-derived JP-8 (and other jet fuels) should be emphasized using liquid chromatograph, GC/MS, and other appropriate techniques.

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5. Scherfig, J., Dixon, P.S. and Justice, C., November 1975. Use of Unicellular Algae for Evaluation of Potential Aquatic Contaminants. Proceedings of the 6th Annual Conference on Environmental Toxicology, December 1975. AFAMRL-TR-75-125. Pages 457-491, Air Force Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio. (AD A-024899)

APPENDIX

A. Preparation of JP-8 Water Soluble Fraction

The water soluble fraction of JP-8 was prepared as follows: 500 mℓ required glass separatory funnels were filled with 380 mℓ of the Standard Algal Assay Medium (100 percent SAAM) and 20 mℓ of shale JP-8 fuel (mixture is 5 percent fuel: 95 percent SAAM). This mixture was shaken for one minute every two hours during an 8-hour period and then allowed to separate for 48 hours. Varying portions of the bottom layer in the funnels (JP-8 soluble fraction) were mixed with regular SAAM to obtain desired concentrations of the water soluble fraction.

Once the flasks were filled with the medium they were seeded with S. capricornutum to an initial concentration of 1.2 to 5 x 10^6 cells/liter. Algal growth was monitored by counting the number of cells, determining total algal volume (biomass) and mean cell volumes on growth days 6, 8, 10 and 12, with the electronic particle counter.

B. Purge and Trap System

A rough flow diagram of the system is shown in Figure 10. The trap consists of stainless steel tubing which contains 60/80 mesh Tenax GC (0.4 g) in the bottom and 100/200 mesh Poropak Q (0.15 g) in the top portion. A 3/8" copper tube wrapped with heating tape is used to heat the trap. A Powerstat variable transformer is connected to the tape and heats the trap to any desired temperature. The following cleansing procedure was used for the trap and all tubing in the system:

- soap and water wash
- deionized water rinse
- acetone wash
- soak in acetone for 24 hours
- dry 200°C for 3 hours
- ultrasonic bath 1 hour
- acetone rinse
- soak in acetone 24 hours
- ultrasonic bath 1 hour
- rinse with 1-2 dichloroethane
- rinse with petroleum ether
- rinse with hexane

C. Gas Chromatography

The column used consists of a 10 percent OV-101, Chrom W-HP 80/100 with a length of 20 feet. The column was conditioned for 72 hours at a constant temperature (200°C), then a mixture of shale JP-8 and hexane was injected into the gas chromatograph and conditions were optimized for best separation of the various components.

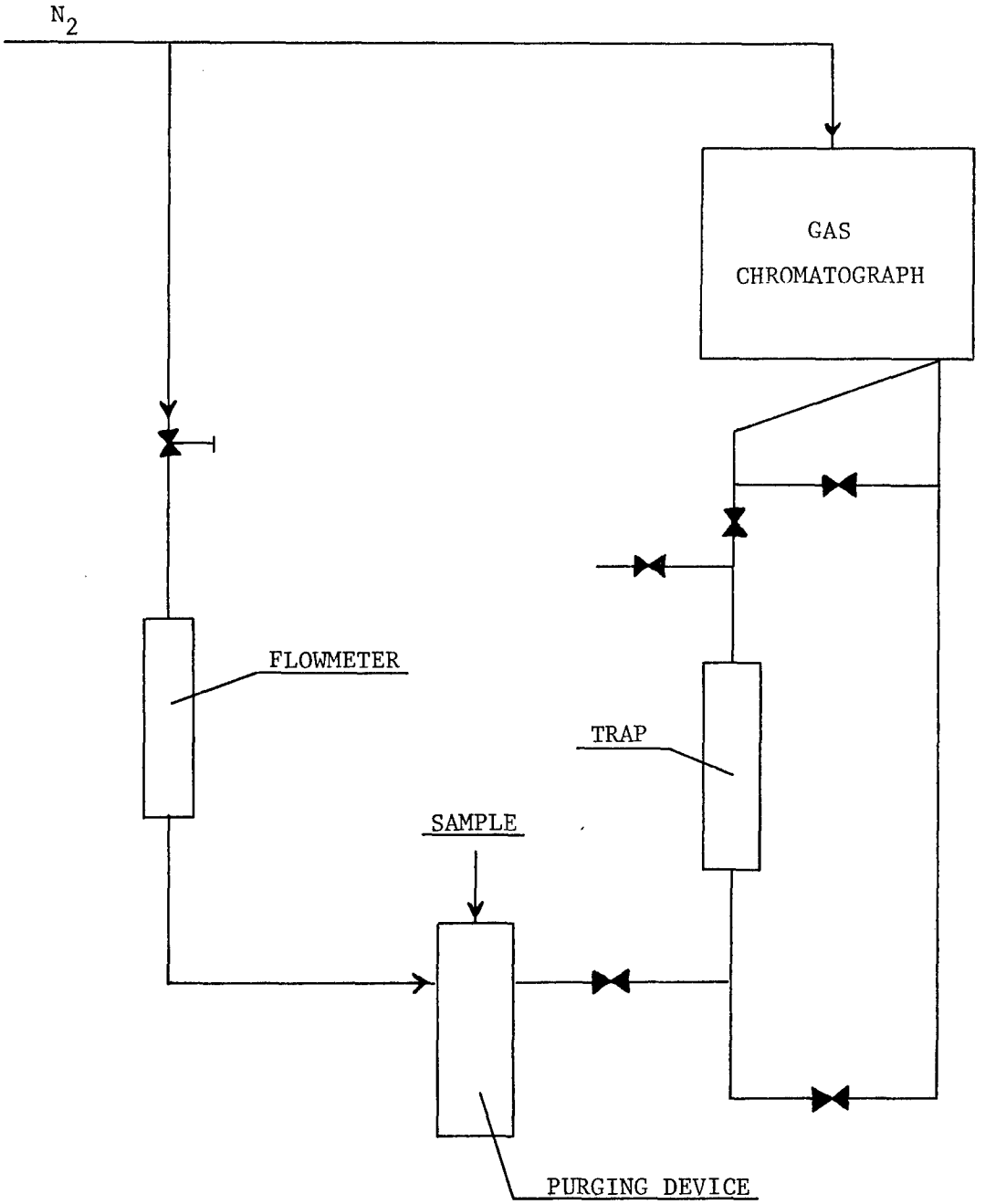

FIGURE 10. DIAGRAM OF PURGE AND TRAP DEVICE

D. Calibrating and Testing the Purge System

Calibration of the purge system includes the heating device for the trap (oven) and the flowmeter for the carrier gas. The former was done by inserting a thermometer into the oven which heats and holds the trap. The flowmeter for the carrier gas was calibrated with a "soap-bubble flowmeter."

This system has been tested with a standard mixture of saturated hydrocarbons ranging from n-hexane to n-pentadecane. These tests were made in order to evaluate the percent recovery obtained by the purge technique. The percent recovery was calculated as the ratio of peak areas obtained by injection into the purge system to peak areas obtained by direct injection into the gas chromatograph.

TABLE 12. GAS CHROMATOGRAPH AND PURGE SYSTEM CONDITIONS

Gas Chromatograph Conditions					
Detector °C	Injector °C	Temp Program in Column	Heating Rate °C/min	Range	Attenuator
250	210	50-180°C	4	1	32
250	210	50-180°C	4	1	32

Purge System			Recorder
Purge Time (min)	Desorption (min)	Chromatogram (min)	Chart Speed in/hr
*	*	60	16
20	10	60	16

*Injected directly into G.C. port

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